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UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010

GUIDE FOR FISH KILL INVESTIGATIONS

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AEHA

This Technical Guide was compiled by:

CARL A. BOUWKAMP
Aquatic Biologist
Water Quality Engineering Division

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER
9 Technical Guide No. 116

2. GOVT ACCESSION NO.
AD-A087143

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)
6 Guide for Fish Kill Investigations Project No. ~~32-24-0193-80~~

5. TYPE OF REPORT & PERIOD COVERED
Technical Report NA

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)
10 Carl A. Bouwkamp

8. CONTRACT OR GRANT NUMBER(s)
12 59

9. PERFORMING ORGANIZATION NAME AND ADDRESS
US Army Environmental Hygiene Agency
Aberdeen Proving Ground, MD 21010

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11 9 May 80

11. CONTROLLING OFFICE NAME AND ADDRESS
US Army Health Services Command
Fort Sam Houston, TX 78234

12. REPORT DATE

13. NUMBER OF PAGES
17

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)
14 USAEHA-[illegible]
USAEHA-[illegible]

15. SECURITY CLASS. (of this report)
UNCLASSIFIED

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
Distribution unlimited.

This document has been approved for public release and sale; its distribution is unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)
Fish kill
Water quality
Water pollution
Algal blooms

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This technical guide was written to serve as an aid in solving fish kills locally by Army installations and give specific guidance on this Agency's role in assisting with fish kill investigations. Possible causes of fish kills, possible preventive measures, and preparing for/conducting fish kill investigations are the major topics discussed.

DD FORM 1473, 1 JAN 73 EDITION OF 1 NOV 65 IS OBSOLETE

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

038150

DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

Mr. Bouwkamp/jg/AUTOVON
584-3814

HSE-EW-A/WP

9 May 1980

SUBJECT: Guide for Fish Kill Investigations, USAEHA Technical Guide No. 116

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Subject document is furnished to serve as an aid to installation level personnel in solving fish kills and gives specific guidance on this Agency's role in assisting with fish kill investigations. Request this Technical Guide be reviewed and forwarded to those activities under your command.

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as

Robert L. Hanson
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U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010

HSE-EW-A/WP Technical Guide

GUIDE FOR FISH KILL INVESTIGATIONS

PREFACE

1. This Agency, due to the nature of its mission and responsibilities in environmental health, receives requests from various Army activities in CONUS and OCONUS for assistance in solving the cause of fish kills. Prior to 23 October 1974 the US Army Environmental Hygiene Agency (USAEHA) had no formal organized approach to handling these kills, and there was no Army activity to which such requests could be adequately referred. Requests for fish kill assistance came by letter or telephone to various USAEHA divisions. More often than not, the division receiving the request did not have the expertise to solve the problem and sought assistance from other divisions. This lack of an organized approach proved unsatisfactory. On 23 October 1974, USAEHA activated the Subhuman Vertebrate Coordinating Committee which is an Agency group that handles requests for assistance in animal kills. Most requests are satisfactorily handled by telephone, and others are referred to more local Army activities that can handle these requests.

2. The Subhuman Vertebrate Coordinating Committee was formed primarily in response to Agency requests for assistance in fish kills. The committee is chaired by a veterinary pathologist and the members include aquatic biologists, entomologists, and chemists from the three Agency divisions that provide analytical support for this committee's activities. It is the policy of this Agency to use a multidisciplined approach when handling fish kills, as expertise in one scientific discipline often does not lend the scope needed to solve the problem at hand. Since the inception of this committee, up to seven fish kills per year have been handled by formal reports. More fish kills have been adequately handled by the use of informal telephone consultations with various committee members and representatives of the requesting organization than through formal Agency studies.

3. This fish kill manual was written and compiled by Mr. Carl Bouwkamp, an aquatic biologist on the staff of this Agency's Water Quality Engineering Division, and edited by the Agency Subhuman Vertebrate Coordinating Committee. This guide was written with the intention that it will serve as an aid in solving fish kills locally by Army installations and gives specific guidance on this Agency's role in assisting with fish kill investigations.

HSE-EW-A/WP Technical Guide
Guide for Fish Kill Investigations

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HSE-EW-A/WP Technical Guide
Guide for Fish Kill Investigations

I. US ARMY ENVIRONMENTAL HYGIENE AGENCY'S ROLE IN FISH KILL INVESTIGATIONS.

A. Support is available through the USAEHA Subhuman Vertebrate Coordinating Committee for investigating Army installation fish kills. Requests for this Agency's assistance should be made by telephone and letter request to: Chief, Pathology and Animal Care Branch, Toxicology Division (Chairman, Subhuman Vertebrate Coordinating Committee), AUTOVON 584-3980, Commercial 301 671-3980, after duty hours AUTOVON 584-3816, Commercial 301 671-3816.

ADDRESS: Commander
US Army Environmental Hygiene Agency
ATTN: HSE-LT, C, PACB
Aberdeen Proving Ground, MD 21010

Requests for assistance should include, when applicable:

1. Fish and Invertebrate Kill Message Form (Appendix A)

2. Fish and Invertebrate Kill Evaluation Form for Field Investigation (Appendix B)

3. The number and size of samples to be submitted

4. The probable number and types of analyses required

5. The date the samples will be received by USAEHA

6. Method of shipment to USAEHA

B. Immediately after USAEHA receives notification of a fish kill with a complete history, the Agency committee with fish kill responsibility meets. At this meeting, decisions are made as to the approach to take to include the appropriate laboratory tests needed. This is why it is absolutely essential that a complete, accurate history of the subject kill be presented to this Agency as soon as possible. Laboratory tests are expensive and time-consuming. Performing unnecessary procedures would be a waste of Army resources.

C. This Agency has laboratory capabilities to perform aquatic bioassays, gross and microscopic pathology evaluations, and chemical evaluations of organic and inorganic pollutants, to include heavy metals, pesticide and herbicide procedures on water, sediment, and biological specimens. USAEHA has a very limited microbiology capability and laboratories that would support fish kill investigations are not arranged to provide a highly restricted chain of custody.

D. Interim and final reports are prepared by the Agency Subhuman Vertebrate Coordinating Committee. This committee is composed of a veterinary pathologist, an aquatic biologist, an entomologist, and two chemists. After receipt of samples, a letter of acknowledgement is sent within 3 days to the contributing organization. Final reports should leave this Agency no later than 45 days after sample receipt. Interim reports are sent when this 45-day deadline cannot be met. Meaningful telephonic contact between the requesting organization and this Agency is encouraged.

E. USAEHA does not routinely provide personnel to requesting agencies, but can on a limited basis, depending on this Agency's judgment and availability of funds. Sample containers can be provided by USAEHA, but usually this is handled at the local level.

F. Once the cause of a kill is known, if applicable, cleanup and preventive measures should be addressed. This Agency has the necessary expertise to consult in this area and assistance should be sought through the Chairman, Subhuman Vertebrate Coordinating Committee (AUTOVON 584-3980, Commercial 301 671-3980).

II. INTRODUCTION.

A. Fish and invertebrates (insects and crustaceans) make excellent water quality monitors. When fish die and float to the surface, it is apparent that all is not well. It is unfortunate that invertebrates are not so apparent to the passive observer as fish are. Invertebrates are generally more sensitive to pollution and would indicate a problem before it becomes so devastating. However, in the event of a fish kill, the condition of the invertebrate population could be very important in narrowing the probable causes. For instance, fish diseases would not affect the invertebrates, whereas pollution would.

B. Man's activities directly or indirectly cause situations that result in water quality problems that can lead to the death of fish or numerous other aquatic organisms. The majority of fish kills can be prevented or their extent greatly reduced by use of a few preventive practices. A thorough, accurate, and timely onsite investigation can greatly increase the ability to determine the cause of a fish kill; thus, making it easier to prevent another occurrence. The possible legal implications and liabilities associated with fish kills are becoming more complex and stringent which also increases the necessity for a thorough and accurate investigation.

C. One of the greatest obstacles to a conclusive investigation of a fish kill is the inability to arrive on the scene soon enough. Speed is of utmost importance in the initial phases of any fish kill investigation. Often, the cause of a kill can never be resolved if the proper data are not collected while the fish are still dying or very shortly thereafter. Toxicants disperse, fish deteriorate, conditions change, fish are blown or drift away from the affected area, and conclusive evidence becomes hard, if not impossible, to find if time is allowed to elapse.

D. Since it is imperative that investigation response be so short and USAEHA has a lack of immediately available personnel, it is recommended that installation personnel carry out the onsite investigation. This guide, plus support furnished by USAEHA, Aberdeen Proving Ground, MD 21010, which provides consultative, analytical, and biological services to Army installations, should be sufficient to determine a cause for most fish kills.

III. OBJECTIVES.

A. To make people aware of the types of data that should be collected in a fish kill investigation.

B. To give guidance on how to be prepared for and to prepare for a fish kill investigation.

C. To give guidance on how to carry out an onsite fish kill investigation.

IV. POSSIBLE CAUSES OF FISH KILLS.

A. Natural Fish Kills.

1. Disease is one natural cause of fish kills. There are certain conditions that must be present for a fish to become diseased or parasitized. Generally, all three must be present for disease to occur:

a. STRESS - may be caused by handling, crowding, low water level, lack of food, excessive noise, turbulence, excessive or sudden change in temperature, pH, or other water quality characteristic.

b. CAUSATIVE AGENT - may be viral, bacterial, or a parasite. There is generally nothing that can be done in nature to control this factor as these agents can be ubiquitous in the aquatic environment.

c. SUSCEPTIBILITY - in many instances the size is very important. Also involved may be the general body condition; i.e., fish are generally weakest in late winter and early spring (spawning) and are more subject to becoming diseased or parasitized. Symptoms exhibited by fish either parasitized or diseased is presented in Appendix C.

2. Algal blooms can cause the following conditions leading to fish kills:

a. One of the most frequent causes of fish kills in ponds and, to a lesser degree, in lakes is algal blooms. Algae are ubiquitous in the aquatic environment. Thus, the only thing preventing algal blooms is the lack of one or more essential requirements for an algal bloom to occur. The primary ingedients for an algal bloom are sufficient nutrients, sunlight, and temperature. Nitrogen and phosphorous are generally the nutrients that limit algal growth in the warm weather months when light and temperature are sufficient. In isolated cases, micronutrients or some other physical condition such as a toxicant, pH, turbidity, or rapid mixing can limit growth.

b. Algae are the primary producers in the aquatic environment, thus producing much of the oxygen and food for the organisms living there. Algae, bacteria, and aquatic organisms all respire and use oxygen. At night, or when algae die, respiration becomes greater than the photosynthetic production of oxygen, and an oxygen deficit can occur. When oxygen diffusion or natural aeration cannot replenish this deficit fast enough, oxygen levels can fall below that required to sustain aquatic life. Certain fish can tolerate lower oxygen levels than others as indicated in Table 1. Under low oxygen conditions, fish can generally be seen gulping air at the surface or lying just under the surface gulping water that is in contact with the air, thus obtaining some oxygen from diffusion.

c. There are generally six ways algal blooms can lead to fish kills.

(1) First, persistent cloudy weather during a bloom condition causes oxygen production through photosynthesis to fall behind the rate of respiration. If the oxygen deficit is great enough, a fish kill occurs.

TABLE 1. LETHAL LEVELS OF DISSOLVED OXYGEN FOR SELECTED FISHES

Scientific Name Common Name	Size	DO mg/L*	Deaths	Temp °C
Aloss sapidissima American shad	6-7 cm	0.9-1.4	50%	21-23
Chaenobryttus gulosus Warmouth	13 cm†	0.4-1.6	100%	21-32
Ctenopharyngodon idella Grass carp	1.8-78 g	0.2-0.6	100% range	12-18
Cyprinus carpio Carp	8 cm 2 yr	0.4-1.2 0.3-0.8	50% 100% range	10-16 5-8
Esox lucius Northern pike	1-2 yr	0.5-1.6	50%	15-25
Ictalurus punctatus Channel catfish	juvenile	0.8-0.9	ave	25-35
Lepomis cyanellus Green sunfish	†	1.5	100%	4
Lepomis gibbosus Pumpkinseed	-- †	3.1 0.9	100% 100%	15 4
Lepomis macrochirus Bluegill	5 cm 2-6 cm†	0.9 0.6-1.1	50% 100%	30 24-30
Micropterus dolomieui Smallmouth bass	4 g	0.5-1.2	50%	11-27
Micropterus Salmoides Largemouth bass	† † 4-14 g	2.3 3.1 0.9-1.4	100% 100% ave 50%	4 15 25-35
Notropis cornutus Common shiner	1-2 yr	0.5-1.0	50%	12-27

See footnotes, page 6.

Scientific Name Common Name	Size	DO mg/L*	Deaths	Temp °C
Oncorhynchus kisutch Coho salmon	Yearling 4-11 cm	1.2-1.6 1.1-1.7	50% 0-83%	14 12-20
Oncorhynchus nerka Sockeye salmon	Adult	2.3-2.7	most	21-23
Perca flavescens Yellow perch	10 cm yearling	0.5-1.2 0.4-0.9	50% 100%	10-20 11-23
Pimephales promelas Fathead minnow	3.6 cm	1.0	none	18-26
Pomoxis nigromaculatus Black crappie	† †	4.3 1.4	100% 100%	26 4
Salmo clarki Cutthroat trout	11-17 cm	1.2-1.4	50%	11
Salmo gairdnerii Rainbow trout	6 mo 10 cm	1.3-1.6 2.4-3.1	50% 50%	13-20 16-20
Salmo solar Atlantic salmon	fingerling yearling	1.5 1.9	threshold threshold	15 16
Salmo trutta Brown trout	yearling 2.9 g	1.5-2.5 3.2	50% 50%	9-21 22-24
Salvelinus fontinalis Brook trout	fingerling yearling	1.0-1.8 1.6-2.6	50% 50%	9 12-21

* From Doudoroff, P. and D. L. Shumway, Dissolved Oxygen Requirements of Freshwater Fishes, Food and Agriculture Organization of the United Nations, Rome, 1970.
† Fish were not allowed access to the surface.

(2) Second, occasionally an algal bloom will experience a rapid die-off rate and the decomposition of algal cells will deplete the oxygen supply.

(3) Third, some forms of algae float to the surface forming a scum layer that impedes light penetration. Thus, photosynthesis only occurs near the surface, and dissolved oxygen (DO) decreases at lower depths where respiration and decomposition are still occurring.

(4) Fourth, scum-forming algae may suffer rapid die offs due to injury sustained from intense sunlight or other causes. Subsequent degradation of algal material causes depletion of dissolved oxygen.

(5) Fifth, algacides are sometimes used to stop an algal bloom, and subsequent decomposition causes oxygen depletion. If use of an algacide is deemed necessary, only a portion of the water body should be treated at a time. Using an algacide is like mowing a lawn; it must be repeated periodically. Generally, it would be better to remove the nutrient source. Treat the ailment rather than the symptom.

(6) Finally, toxins produced by certain species of algae will sometimes cause a fish kill. Generally, this phenomenon occurs in the marine environment with dinoflagellates. However, toxicity occasionally occurs in fresh water and is generally caused by the breakdown products of proteins contained in blue-green algae.

d. There are a few characteristics one can look for in determining if an algal bloom could have caused a fish kill. Discoloration of the water, other than silt load, may indicate a bloom. Most blooms will give a greenish color that is often described as a "pea soup" green. However, some species of Anabaena cause a bright blue color, while species of Trachelomonas may cause a reddish to brown color. Generally, when such conditions exist and an object cannot be distinguished more than a few inches into the water, a bloom could be occurring. DO and pH will go high (DO of 10 mg/L or above, pH 10 or above) during midday, and both will drop substantially during the night, reaching a low about daybreak (DO 0-5 mg/L, pH 5-7).

e. If an algal bloom is suspect for a fish kill, one should try to locate the source of the nutrients. Water samples of any point discharge should be collected and analyzed for nutrients (Appendix D). Other possible sources may be agricultural, golf course or lawn runoff, intentional fertilization of ponds for fish production, septic tank leachate, or tributary streams that receive sewage treatment effluent or other nutrient-rich water.

f. Many times, the source of nutrients can be determined during the investigation. If a certain source is suspect, a water sampling scheme should be implemented to confirm or disprove the suspicion (paragraph VII.D). In cases where an apparent source of nutrients cannot be found, samples should be collected from influent streams to pinpoint the area from which the nutrients originate.

g. When an algal bloom is suspect for a fish kill, a representative algal sample should be collected (paragraph VII.E). Very often even a representative sample will not help the phycologist to be conclusive. In most fish kills, notification of the kill to the proper authorities comes so late that comprehensive algal analyses become futile. Nevertheless, only with representative algal samples can the phycologist have the opportunity to confirm an algal bloom as the causative agent of a fish kill.

h. A representative algal sample should not be collected where algae have accumulated because of wind action. It should be collected below the surface with no clumps of surface algae. If surface algae are suspect for a kill, a separate sample should be taken of the surface scum. A liter of water collected per site is sufficient for a sample.

i. The presence of a species of algae known to be toxic is not proof it was the causative agent, nor are high numbers of algae proof that the algae depleted the oxygen. The oxygen should be measured at dawn and, if levels are sufficient (4-5 ppm for warmwater fish), testing for toxicity could be performed. One could place some unaffected fish in a tank of oxygenated water that previously killed fish to see if they survive. Even a bioassay will not prove that toxic algae killed the fish; but identification of toxic algae in large numbers along with the bioassay would be rather strong evidence.

j. Algae are normally not a problem in a river system. Most of the algae are attached. The nature of flowing water is such that plankton does not become abundant. Also, with the turbulence of the water, a larger portion of oxygen can be provided by aeration. Streams can handle a higher biochemical oxygen demand (BOD) loading than standing water. Thus, unless a stream is moving very slowly or a high BOD loading is added to the stream, oxygen depletion will not occur. However, discharges are normally in streams, and high BOD loading can occur.

3. Oxygen depletion due to ice and snow cover can be another cause for fish kills. At low temperatures, water can hold much more oxygen, and respiration is greatly slowed. But when ice forms, surface aeration can no longer provide any oxygen to the system. The oxygen present at the time of ice formation, plus what is produced in photosynthesis and any oxygenated water entering the system, must last until spring breakup. If the ice and

snow cover reduce light penetration enough to slow photosynthesis so that less oxygen is produced than respired, oxygen concentration can fall. If oxygen is depleted to the point where fish can no longer survive, there is a fish kill. The snow and ice must be very thick, the water shallow, and the ice cover prolonged for such a kill to occur.

4. Oxygen depletion or pH changes due to plant respiration or organic decomposition can be a contributing factor in fish kills. The decomposition of organic matter demands alot of oxygen and lowers the pH. The fish would normally die from oxygen depletion. However, the lower pH would contribute to the stress on the fish.

5. Abrupt temperature changes do not occur very often in natural waters, but thermal effluent and reservoir releases could be a problem leading to fish kills. Naturally, a seiche could bring cold water to the surface that could cause a temperature change of several degrees. Oxygen depletion in the hypolimnion could drive coldwater fish into surface waters that are too warm for their survival. Fish have a tolerance level above which they cannot survive. Table 2 gives some temperature criteria for selected fish.* Values vary considerably according to acclimation temperature or whether or not the fish are under some additional stress.

6. Spring or fall turnover can bring toxic materials or oxygen-free water to the surface causing a kill. This type of fish kill will happen when the water is a uniform temperature throughout, and wave action brings hypolimnetic water to the surface. Many toxic materials become more soluble in a reducing (oxygen-deficient) environment.

7. High winds can cause a seiche movement in which toxic or DO-free hypolimnetic water is brought to the surface even against thermal density gradients leading to a fish kill. The seiche could cause temperature or salinity changes also.

8. Salinity changes can also cause fish kills. Large quantities of rain or long periods without rain can cause such changes. In estuaries where this generally occurs, the fish normally move with the change and avoid problems. However, fish are sometimes restricted in their movement or changes occur too rapidly.

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* Brungs, W. A. and B. R. Jones, Temperature Criteria for Freshwater Fish: Protocol and Procedures, US Environmental Protection Agency (EPA) Publication 600/3-77-061, 1977.

TABLE 2. TEMPERATURE CRITERIA FOR GROWTH AND SURVIVAL OF FISH. [° C (° F)]

Species	Maximum weekly average temperature for growth[a]	Maximum temperature for survival of short exposure[b]
Alewife	—	—
Atlantic salmon	20 (68)	23 (73)
Bigmouth buffalo	—	—
Black crappie	27 (81)	—
Bluegill	32 (90)	35 (95)
Brook trout	19 (66)	24 (75)
Brown bullhead	—	—
Brown trout	17 (63)	24 (75)
Carp	—	—
Channel catfish	32 (90)	35 (95)
Coho salmon	18 (64)	24 (75)
Emerald shiner	30 (86)	—
Fathead minnow	—	—
Freshwater drum	—	—
Lake herring (cisco)	17 (63)[c]	25 (77)
Lake whitefish	—	—
Lake trout	—	—
Largemouth bass	32 (90)	34 (93)
Northern pike	28 (82)	30 (86)
Pumpkinseed	—	—
Rainbow smelt	—	—
Rainbow trout	19 (66)	24 (75)
Sauger	25 (77)	—
Smallmouth bass	29 (84)	—
Smallmouth buffalo	—	—
Sockeye salmon	18 (64)	22 (72)
Striped bass	—	—
Threadfin shad	—	—
Walleye	25 (77)	—
White bass	—	—
White crappie	28 (82)	—
White perch	—	—
White sucker	28 (82)[c]	—
Yellow perch	29 (84)	—

[a]Calculated according to equation:
maximum weekly average temperature for growth = optimum for growth + (1/3) (ultimate incipient lethal temperature - optimum for growth).

[b]Based on: temperature (° C) = (log time (min) - a)/b - 2° C, acclimation at the maximum weekly average temperature for summer growth

[c]Based on data for larvae.

9. Severe storms, water level fluctuations, turbidity, siltation, or runoff can also cause fish kills.

10. Physiological changes such as spawning can cause fish kills. Salmon, Alewives, and shad are often found after spawning.

11. Fish can also die of old age, but the numbers affected at any one time are usually small and normally occur under stressful conditions.

B. Man-induced Fish Kills.

1. Industrial wastewater discharges could contain a wide range of toxic substances. Some of the toxicants could be metal-plating wastes, ammunition or explosive wastes, solvents, grease and oils, acidic or alkaline wastes, photographic wastes, organic compounds, pesticides, or Polychlorinated Biphenyls (PCB) to name a few. Also industrial wastes can have a high BOD or chemical oxygen demand (COD), causing oxygen depletion.

2. Domestic wastewater discharges could contain a wide variety of toxicants, especially if industrial wastewater goes to the sewage treatment plant. However, domestic wastewater normally contributes nutrients (nitrogen and phosphorous), detergents, BOD and, if chlorinated, some toxic chlorine and chloramines. Nutrients can cause eutrophication, algal blooms and, eventually, oxygen depletion. Detergents disrupt gill tissue and oxygen transfer. A BOD greater than the assimilation capacity of the receiving water can also cause oxygen depletion. Chlorine can be toxic at very low levels. In EPA Quality Criteria for Water,* it is recommended that total residual chlorine not exceed 0.002 mg/L for salmon and 0.01 mg/L for other aquatic life.

3. Agriculture and related activities can cause fish kills through poor control of pesticides, fertilizers, or organic waste products. Most contributions from agriculture would be in the runoff. However, direct contamination is highly possible. Spraying of ditch banks, pond edges, or wind drift of sprays into the water can cause direct contamination. Also, pesticide containers that are rinsed out, discarded, or used for floats in water bodies can cause fish kills. Fertilizers and organic wastes can cause problems similar to those of domestic wastewater discharges.

4. Temporary activities such as pesticide spraying, construction, and spills should be considered in the event of a fish kill. Army

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* Quality Criteria for Water, Document No. EPA-440/9-76-023, 1976.

installations generally have an extensive spraying program, have denuded areas for several different reasons, and have large quantities of potentially dangerous compounds stored or shipped.

5. Water manipulation such as dams can cause fish kills. If hypolimnetic water is released, it could possibly be oxygen-deficient, too cold, toxic, or too high in carbon dioxide. Water falling over a dam and allowed to entrap air that is then pulled to great depths will become supersaturated with gases causing gas-bubble disease in fish commonly called "pop eye" disease. Water manipulation can also stop migration and spawning, and alter habitat conditions so that fish populations could drastically change or be eliminated without physically killing a fish.

6. Other possible causes of fish kills could be explosions, abrupt water-level fluctuations, extreme turbidity, or siltation. Also, it may not always be just one factor, but a combination of stresses, that add up to a mortality. If a water body lies near an impact area, be sure to check previous firing schedules and the possibility of explosions.

V. POSSIBLE PREVENTIVE MEASURES. It is the responsibility of the installation to prevent man-caused fish kills. If a fish kill gets off the installation, the commander could be held legally and monetarily responsible for damages due to negligence. To prevent such a situation, there are a few precautions that should be taken.

A. Be sure that all wastewater is properly treated before discharge or proper corrective measures have been taken.

B. Be sure there is an adequate spill prevention program and stress the need for immediate reporting of accidental releases or spills of potentially toxic or hazardous materials.

C. Be sure there is an adequate cleanup plan, and that it is implemented in a timely manner. This plan should include notification of the office responsible for fish kill investigations so that advanced preparation for an investigation can be accomplished.

D. Be sure the pesticides-spraying program has adequate precautions against contaminating surface waters either directly or through runoff.

E. Try to avoid having recreational reservoirs that receive discharges or nutrient loading. Also, whenever possible, have discharge outfalls in large streams that have large dilution and assimilation capacities.

F. Implement these preventive measures through an active and vigorous base-wide education program.

VI. PREPARING FOR A FISH KILL INVESTIGATION.

A. There is always the possibility of legal liability associated with a fish kill. Thus, the investigator's report may be subject to the scrutiny of judge and jury. Both planning and conduct of the investigation must be done with great care. A carefully-developed, routine field procedure should be available for immediate activation whenever a fish kill is reported.

B. Speed is of the utmost importance in a conclusive investigation. One should collect as much information as feasible while the fish are still dying or as soon as possible thereafter. One valuable source of information is the informant. He was the first to observe the dead or dying fish and, thus, could be very helpful in the investigation. One should fill out a Fish and Invertebrate Kill Message Form (Appendix A) as completely as possible before the informant has a chance to get away. It would also be very helpful if the informant could participate in the field investigation. Much time could be saved in locating the kill and answering questions.

C. The next step is to develop a plan for this particular kill. Secure maps of the area to be investigated. US Geological Survey maps are best if available. Otherwise, use the most detailed map available. Determine the area of the fish kill and access points to be used. Also locate possible industrial, municipal, agricultural or other possible sources of pollution. Determine the type and number of samples to be taken, how the logistics will be handled, and what transportation will be needed.

D. An in-depth study of a fish kill requires equipment and qualified personnel. However, the need for quick response makes it necessary to be ready in advance and make do with people and resources available. A check list of equipment is presented in Table 3. If personnel and equipment are not available for an in-depth study, do as thorough a job as possible. If USAEHA assistance is required, see paragraph I (USAEHA Role in Fish Kill Investigations) of this manual.

VII. FIELD INVESTIGATION.

A. Have the proper people onsite and inform the proper authorities. Invite the informant to accompany the investigation team. The information he may possess could be very helpful. If the commander so deems it, a representative should be informed and invited from the State agency in charge of fisheries and/or water pollution control. Take along the Fish and Invertebrate Kill Evaluation Form for Field Investigation (Appendix B) and complete it onsite.

TABLE 3. CHECKLIST OF EQUIPMENT FOR FISH KILL INVESTIGATIONS

General

1. Maps
2. DO meter or kit
3. pH meter or kit
4. Thermometer
5. Water Sampler
6. Sample containers (Appendix D)
7. Ice chests or insulated containers
8. Wet ice
9. Dry ice
10. Waders
11. Boat
12. Motor
13. Paddles
14. Life preservers
15. Waterproof notebook
16. Waterproof labels
17. Waterproof marker
18. Portable light source
19. Paper towels
20. Aluminum foil
21. Insulated shipping containers
22. Plastic bags, assorted sizes
23. Camera
24. Film

Fish

1. Dipnets
2. Seines
3. Nets
4. Rake
5. Tubs
6. Weight scale
7. Measuring board
8. Fish counting forms
9. Dissecting kit
10. Formalin
11. Scale envelopes

Benthos

1. Dredge sampler
2. Surber sampler
3. Drift net sampler
4. Kicknets
5. Quart or pint widemouth containers
6. 95-percent alcohol
7. Sieves

Plankton

1-quart jars

B. Type and Extent of Fish Kill.

1. Make a reconnaissance of the kill area to get a feel for what may have caused the kill, how extensive the kill is, and whether it is, indeed, a kill. A fish kill can be minor (1-100 fish), moderate (100-1000 fish), or major (1000 fish and above). If a kill is so large that counting all the dead fish is not feasible, an estimate must be made. Estimates obtained using the following procedures will be conservative and very seldom represent more than a fraction of the fish killed. These estimates are based solely on the number of fish visible at a point in time. Many may not be visible because they are not floating, hidden by debris, blown or drifted away, taken by scavengers, decomposed, not yet dead, or overlooked (human error).

2. When subsampling to estimate the number of dead fish, bias may be introduced. In order to produce unbiased results, certain sample principles must be followed.

a. The fish kill area is divided into smaller areas (units) in which the number of dead fish are counted and the number expanded to represent the total area.

b. These sample units must be chosen at random.

c. Precision depends on sample size. The more units counted, the more precise the estimate will be.

3. Counting procedures for streams and lakes, as presented by the South Carolina Department of Health and Environmental Control,* are presented in Appendix E.

C. Try to pinpoint the possible cause or causes of the fish kill.

1. General observations of the behavior, condition, location, and kinds of organisms dying; water conditions; weather conditions; discharge locations; and any other pertinent information can help narrow the possibilities.

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* Division of Biological and Special Services, Manual for Fish Kill Investigations, South Carolina Department of Health and Environmental Control, Bureau of Field and Analytical Services, 1979.

2. General water quality data such as DO, pH, temperature, and conductivity can also be useful tools in determining the direction of the investigation. Anything that will eliminate possibilities can lessen the extent of the investigation.

D. Collect water and sediment samples for chemical and pesticide analyses.

1. Map out a sampling plan that will maximize the amount of information for the number of samples. The water and sediment samples should be from the same locations as DO, pH, temperature, and conductivity. While it is better to have too many samples than too few, an effort should be made not to overload the laboratory with samples because of poor sampling procedures. A sample should be collected both inside and outside the kill area. Any point discharge that may be suspect in the kill should have the outfall sampled along with any other samples that would be needed to prove that particular discharge did or did not cause the fish kill. With a discharge to a stream, one sample should be collected above the outfall, one at the outfall, one far enough below the outfall for mixing, and one far enough downstream to be out of the kill area. With a discharge to a lake, samples must be taken at increasing distances from the outfall, with one outside the kill area. Take into consideration possible currents, especially an estuary or large lake.

2. After contacting Coordinating Chairman (paragraph IA), consult with Chief, Water and Waste Chemistry Branch regarding chemistry (AUTOVON 584-2208, Commercial 301 671-2208) and Chief, Pesticide Monitoring Branch regarding pesticides and PCB's (AUTOVON 584-3613, Commercial 301 671-3613) before collecting the samples, unless doing so would cause an untimely delay. They will give you insight into what samples are needed and how much water and sediment would be needed for your particular situation. Sample collection and preservation methods are presented in Appendix D. Because many chemical parameters must be analyzed shortly after collection, and most installations have a laboratory, it is encouraged that the installation do whatever parameters they have the capability for. This Agency can supply any additional support needed. The water and sediment samples for pesticide and PCB analyses should be collected in 1-liter, glass bottles with Teflon® cap liner or aluminum foil (dull side to sample). The bottles should be rinsed with pH-2 sulfuric acid water, rinsed thoroughly with distilled water, acetone-rinsed, allowed to air dry, and then capped. In all sampling, be sure containers are well labeled with permanent ink and labels.

® Teflon is a registered trademark of E. I. DuPont de Nemours and Company, Wilmington, DE. Use of trademarked name does not imply endorsement by the US Army, but is intended only to assist in identification of a specific product.

E. Biological Samples.

1. Collect Biological Samples. The extent of the fish kill will help determine the number of organisms needed for a representative sample. In most cases, 10 individuals of each species should be collected. If the kill affects fewer than 10 organisms per species, collect all affected. Never collect decomposed fish. Collect dying fish whenever possible or fish with pink still left in their gills. Use good judgment in collecting organisms whether fish, aquatic insects, crayfish, clams, etc. The larger the organism, the smaller the number needed to make a representative sample. Organisms should be collected as soon as possible, wrapped in aluminum foil with the dull side toward the sample, placed inside plastic bags or other containers, and frozen as soon as possible. The process should be repeated collecting samples from outside the kill area but within the same body of water, if possible. This will be much more difficult since the organisms will still be alive and hard to capture. Seines, gill nets, trammel nets, traps, trawls, electrofishing, trot lines, or other devices may be used for fish, and nets, dredges, and sieves for invertebrates. Collect 1 liter of water for plankton, 2 gallons for bioassay; add no preservative; and freeze allowing head space for expansion and resuspension.

2. Biological samples can also be submitted to this Agency for identification of species. These fish or invertebrates can be preserved in 10-percent formalin or 70-percent alcohol and shipped with the water samples.

F. Shipment of Samples. Before anything is sent, be sure all samples are marked as to sample type, preservative, filtered or unfiltered for water chemistries, location (sample site designation), installation, collector, date and time of collection, and analyses to be performed. All samples should be logged, a copy retained, and a copy sent with the samples. Also, separately mail another copy of the log sheet and a map showing kill area and sampling locations along with copies of the Fish and Invertebrate Kill Message Form (Appendix A) and the Fish and Invertebrate Kill Evaluation Form for Field Investigation (Appendix B). All frozen samples should be packed on dry ice and clearly marked "Frozen Specimens Packed on Dry Ice." All others can be packed on wet ice or divided as to samples requiring refrigeration and those not. Be sure labels will not become illegible or unglued in water. Samples should be sent on a Government Bill of Lading (GBL) by air express. Before shipping, contact Chief, Pathology and Animal Care Branch (AUTOVON 584-3980, Commercial 301 671-3980) and provide the name of the airline, the flight number, and the estimated time of arrival.

APPENDIX A

FISH AND INVERTEBRATE KILL MESSAGE FORM

FISH AND INVERTEBRATE KILL MESSAGE FORM
(USAEHA TECHNICAL GUIDE 116)

MESSAGE RECIPIENT DATA

NAME		ORGANIZATION
DATE	TIME	MEANS OF CONTACT

INFORMANT DATA

NAME	ADDRESS
TELEPHONE: *(WORK)*	*(HOME)*

KILL LOCATION

COUNTY	STATE	WATER BODY
DESCRIPTION OF HOW TO LOCATE KILL AREA		

OBSERVATIONS

DATE OBSERVED	TIME OBSERVED	KINDS OF ORGANISMS AFFECTED

RELATIVE NUMBERS: ☐ 1-100 MINOR ☐ 100-1000 MODERATE ☐ 1000 OR MORE MAJOR

SIZE RANGE	WATER CLARITY: ☐ VERY CLEAR ☐ CLEAR ☐ TURBID ☐ HIGHLY TURBID

ANY UNUSUAL COLORATION *If yes, state color*		NO		YES
ANY VISIBLE SIGNS OF INJURY OR DISEASE? *(i.e. fungus, cyst)* *If yes, describe condition*		NO		YES
WERE ORGANISMS STILL DYING? *If no, proceed to WEATHER CONDITIONS*		NO		YES
ARE FISH SWIMMING WILDLY?		NO		YES
HAVE THE FISH LOST THEIR EQUILIBRIUM?		NO		YES
ARE THE FISH LETHARGIC?		NO		YES
ANY UNUSUAL BREATHING RATE? *(i.e. rapid, slow)* *If yes, describe*		NO		YES

COMMENTS:

WEATHER CONDITIONS

PRECIPITATION *(amount)*	SKY *(percent cloud cover)*	AIR TEMPERATURE

WIND DIRECTION	WIND VELOCITY

PRIOR WEATHER CONDITIONS *(3-4 days)*

SPECIAL CONDITIONS *(i.e. tide, drought, flood, hurricane, hot spell)*

GENERAL DATA

POSSIBLE CAUSES OF FISH KILL

COMMENTS:

AEHA Form 30, 1 Apr 80 (HSE-EW)

FISH AND INVERTEBRATE KILL MESSAGE FORM

(USAEHA TECHNICAL GUIDE 116)

MESSAGE RECIPIENT DATA

NAME		ORGANIZATION
DATE	TIME	MEANS OF CONTACT

INFORMANT DATA

NAME	ADDRESS
TELEPHONE: *(WORK)*	*(HOME)*

KILL LOCATION

COUNTY	STATE	WATER BODY
DESCRIPTION OF HOW TO LOCATE KILL AREA		

OBSERVATIONS

DATE OBSERVED	TIME OBSERVED	KINDS OF ORGANISMS AFFECTED

RELATIVE NUMBERS: ☐ 1-100 MINOR ☐ 100-1000 MODERATE ☐ 1000 OR MORE MAJOR

SIZE RANGE

WATER CLARITY: ☐ VERY CLEAR ☐ CLEAR ☐ TURBID ☐ HIGHLY TURBID

ANY UNUSUAL COLORATION *If yes, state color*		NO		YES
ANY VISIBLE SIGNS OF INJURY OR DISEASE? *(i.e. fungus, cyst)* *If yes, describe condition*		NO		YES
WERE ORGANISMS STILL DYING? *If no, proceed to WEATHER CONDITIONS*		NO		YES
ARE FISH SWIMMING WILDLY?		NO		YES
HAVE THE FISH LOST THEIR EQUILIBRIUM?		NO		YES
ARE THE FISH LETHARGIC?		NO		YES
ANY UNUSUAL BREATHING RATE? *(i.e. rapid, slow)* *If yes, describe*		NO		YES

COMMENTS:

WEATHER CONDITIONS

PRECIPITATION *(amount)*	SKY *(percent cloud cover)*	AIR TEMPERATURE

WIND DIRECTION	WIND VELOCITY

PRIOR WEATHER CONDITIONS *(3-4 days)*

SPECIAL CONDITIONS *(i.e. tide, drought, flood, hurricane, hot spell)*

GENERAL DATA

POSSIBLE CAUSES OF FISH KILL

COMMENTS:

AEHA Form 30, 1 Apr 80 (HSE-EW)

FISH AND INVERTEBRATE KILL MESSAGE FORM

(USAEHA TECHNICAL GUIDE 116)

MESSAGE RECIPIENT DATA

NAME		ORGANIZATION
DATE	TIME	MEANS OF CONTACT

INFORMANT DATA

NAME	ADDRESS
TELEPHONE: *(WORK)*	*(HOME)*

KILL LOCATION

COUNTY	STATE	WATER BODY
DESCRIPTION OF HOW TO LOCATE KILL AREA		

OBSERVATIONS

DATE OBSERVED	TIME OBSERVED	KINDS OF ORGANISMS AFFECTED

RELATIVE NUMBERS: ☐ 1-100 MINOR ☐ 100-1000 MODERATE ☐ 1000 OR MORE MAJOR

SIZE RANGE	WATER CLARITY: ☐ VERY CLEAR ☐ CLEAR ☐ TURBID ☐ HIGHLY TURBID

ANY UNUSUAL COLORATION *If yes, state color*		NO		YES
ANY VISIBLE SIGNS OF INJURY OR DISEASE? *(i.e. fungus, cyst)* *If yes, describe condition*		NO		YES
WERE ORGANISMS STILL DYING? *If no, proceed to WEATHER CONDITIONS*		NO		YES
ARE FISH SWIMMING WILDLY?		NO		YES
HAVE THE FISH LOST THEIR EQUILIBRIUM?		NO		YES
ARE THE FISH LETHARGIC?		NO		YES
ANY UNUSUAL BREATHING RATE? *(i.e. rapid, slow)* *If yes, describe*		NO		YES

COMMENTS:

WEATHER CONDITIONS

PRECIPITATION *(amount)*	SKY *(percent cloud cover)*	AIR TEMPERATURE
WIND DIRECTION	WIND VELOCITY	

PRIOR WEATHER CONDITIONS *(3-4 days)*

SPECIAL CONDITIONS *(i.e. tide, drought, flood, hurricane, hot spell)*

GENERAL DATA

POSSIBLE CAUSES OF FISH KILL

COMMENTS:

AEHA Form 30, 1 Apr 80 (HSE-EW)

FISH AND INVERTEBRATE KILL MESSAGE FORM

(USAEHA TECHNICAL GUIDE 116)

MESSAGE RECIPIENT DATA

NAME		ORGANIZATION
DATE	TIME	MEANS OF CONTACT

INFORMANT DATA

NAME	ADDRESS
TELEPHONE: *(WORK)*	*(HOME)*

KILL LOCATION

COUNTY	STATE	WATER BODY
DESCRIPTION OF HOW TO LOCATE KILL AREA		

OBSERVATIONS

DATE OBSERVED	TIME OBSERVED	KINDS OF ORGANISMS AFFECTED

RELATIVE NUMBERS: ☐ 1-100 MINOR ☐ 100-1000 MODERATE ☐ 1000 OR MORE MAJOR

SIZE RANGE

WATER CLARITY: ☐ VERY CLEAR ☐ CLEAR ☐ TURBID ☐ HIGHLY TURBID

ANY UNUSUAL COLORATION *If yes, state color*		NO		YES
ANY VISIBLE SIGNS OF INJURY OR DISEASE? *(i.e. fungus, cyst)* *If yes, describe condition*		NO		YES
WERE ORGANISMS STILL DYING? *If no, proceed to WEATHER CONDITIONS*		NO		YES
ARE FISH SWIMMING WILDLY?		NO		YES
HAVE THE FISH LOST THEIR EQUILIBRIUM?		NO		YES
ARE THE FISH LETHARGIC?		NO		YES
ANY UNUSUAL BREATHING RATE? *(i.e. rapid, slow)* *If yes, describe*		NO		YES

COMMENTS:

WEATHER CONDITIONS

PRECIPITATION *(amount)*	SKY *(percent cloud cover)*	AIR TEMPERATURE

WIND DIRECTION	WIND VELOCITY

PRIOR WEATHER CONDITIONS *(3-4 days)*

SPECIAL CONDITIONS *(i.e. tide, drought, flood, hurricane, hot spell)*

GENERAL DATA

POSSIBLE CAUSES OF FISH KILL

COMMENTS:

AEHA Form 30, 1 Apr 80 (HSE-EW)

FISH AND INVERTEBRATE KILL MESSAGE FORM

(USAEHA TECHNICAL GUIDE 116)

MESSAGE RECIPIENT DATA

NAME		ORGANIZATION
DATE	TIME	MEANS OF CONTACT

INFORMANT DATA

NAME	ADDRESS
TELEPHONE: *(WORK)*	*(HOME)*

KILL LOCATION

COUNTY	STATE	WATER BODY
DESCRIPTION OF HOW TO LOCATE KILL AREA		

OBSERVATIONS

DATE OBSERVED	TIME OBSERVED	KINDS OF ORGANISMS AFFECTED

RELATIVE NUMBERS: ☐ 1-100 MINOR ☐ 100-1000 MODERATE ☐ 1000 OR MORE MAJOR

SIZE RANGE

WATER CLARITY: ☐ VERY CLEAR ☐ CLEAR ☐ TURBID ☐ HIGHLY TURBID

ANY UNUSUAL COLORATION *If yes, state color*		NO		YES
ANY VISIBLE SIGNS OF INJURY OR DISEASE? *(i.e. fungus, cyst)* *If yes, describe condition*		NO		YES
WERE ORGANISMS STILL DYING? *If no, proceed to WEATHER CONDITIONS*		NO		YES
ARE FISH SWIMMING WILDLY?		NO		YES
HAVE THE FISH LOST THEIR EQUILIBRIUM?		NO		YES
ARE THE FISH LETHARGIC?		NO		YES
ANY UNUSUAL BREATHING RATE? *(i.e. rapid, slow)* *If yes, describe*		NO		YES

COMMENTS:

WEATHER CONDITIONS

PRECIPITATION *(amount)*	SKY *(percent cloud cover)*	AIR TEMPERATURE

WIND DIRECTION	WIND VELOCITY

PRIOR WEATHER CONDITIONS *(3-4 days)*

SPECIAL CONDITIONS *(i.e. tide, drought, flood, hurricane, hot spell)*

GENERAL DATA

POSSIBLE CAUSES OF FISH KILL

COMMENTS:

AEHA Form 30, 1 Apr 80 (HSE-EW)

APPENDIX B

FISH AND INVERTEBRATE KILL EVALUATION FORM FOR FIELD INVESTIGATION

FISH AND INVERTEBRATE KILL EVALUATION FORM FOR FIELD INVESTIGATION
(USAEHA TECHNICAL GUIDE 116)

FISH AND INVERTEBRATE KILL EVALUATION FORM FOR FIELD INVESTIGATION (USAEHA TECHNICAL GUIDE 116)	INVESTIGATION DATE	INVESTIGATION TIME

INVESTIGATOR *(name)*

ORGANIZATION

AGENCIES NOTIFIED

FIELD REPRESENTATIVES PARTICIPATING FROM VARIOUS AGENCIES *(names)*

LOCATION

MAJOR WATER BODY AFFECTED	TRIBUTARIES INVOLVED	AREA AFFECTED	
		ACRES	RIVER MILES

GENERAL DESCRIPTION OF AREA

WATER CONDITIONS *(within KILL Zone)*

WATER CLARITY	UNUSUAL COLORATION	DEPTH	
VERY CLEAR ☐ CLEAR ☐ TURBID ☐ HIGHLY TURBID ☐	☐ NO ☐ YES *(state color)*	MEAN	MAXIMUM

UNUSUAL APPEARANCE *(i.e. algal blooms, oil, turbid)*

TIDAL DATA

PROFILES

	DAWN AM						MIDDAY PM				
DEPTHS	SURFACE				BOTTOM		SURFACE				BOTTOM
TEMPERATURE											
pH											
SALINITY (CONDUCTANCE)											
DISSOLVED OXYGEN (mg/L)											

WEATHER CONDITIONS

PRECIPITATION *(amount)*	SKY *(percent cloud cover)*	AIR TEMPERATURE	WIND DIRECTION	VELOCITY

PRIOR WEATHER CONDITIONS *(3-4 days)*

SPECIAL CONDITIONS *(i.e. tide, drought, flood, hurricane, hot spell)*

OTHER COMMENTS

AEHA Form 29, 1 Apr 80 (HSE-EW)

FISH AND INVERTEBRATE OBSERVATIONS

		NO		YES
ANY VISIBLE SIGNS OF INJURY OR DISEASE? *(i.e. fungus, cyst)* *If yes, describe condition*		NO		YES
ARE ORGANISMS DYING AT PRESENT TIME?		NO		YES
ARE FISH SWIMMING WILDLY?		NO		YES
HAVE THE FISH LOST THEIR EQUILIBRIUM?		NO		YES
ARE THE FISH LETHARGIC?		NO		YES
ANY UNUSUAL BREATHING RATE? *(i.e. rapid, slow)* *If yes, describe*		NO		YES

CONDITION OF GILLS *(describe)*

INTERNAL ABNORMALITIES *(describe)*

KILL ESTIMATE

SPECIES	EST. NO. KILLED	SIZE RANGE	AVE. SIZE	AVE. WEIGHT	EST. TOTAL WEIGHT	COMMENTS

DURATION OF KILL: ☐ IN PROGRESS ☐ FINAL DETERMINATION (days, hours)

SUSPECTED CAUSE OF KILL:

☐ NATURAL ☐ MAN-CAUSED *(specify below)*

☐ AGRICULTURAL ☐ INDUSTRIAL ☐ MUNICIPAL ☐ OTHER

SPECIFIC AGENT OR CAUSE *(if known)*:

TYPE OF SAMPLES COLLECTED [*check appropriate box(es)*]

WATER SAMPLES	☐ SEDIMENT SAMPLES	BIOLOGICAL SAMPLES
☐ BIOASSAY (2 GAL) ☐ CHEMISTRY ☐ PESTICIDES		☐ FISH AND INVERTEBRATE CARCASSES ☐ PLANKTON

CERTIFIED BY *(signature and date)*

FISH AND INVERTEBRATE KILL EVALUATION FORM FOR FIELD INVESTIGATION *(USAEHA TECHNICAL GUIDE 116)*	INVESTIGATION DATE	INVESTIGATION TIME

INVESTIGATOR *(name)*	ORGANIZATION

AGENCIES NOTIFIED

FIELD REPRESENTATIVES PARTICIPATING FROM VARIOUS AGENCIES *(names)*

LOCATION

MAJOR WATER BODY AFFECTED	TRIBUTARIES INVOLVED	AREA AFFECTED	
		ACRES	RIVER MILES

GENERAL DESCRIPTION OF AREA

WATER CONDITIONS *(within KILL Zone)*

WATER CLARITY	UNUSUAL COLORATION	DEPTH	
VERY CLEAR ☐	☐ NO	MEAN	MAXIMUM
CLEAR ☐	☐ YES *(state color)*		
TURBID ☐			
HIGHLY TURBID ☐			

UNUSUAL APPEARANCE *(i.e. algal blooms, oil, turbid)*

TIDAL DATA

PROFILES

	DAWN ___ AM						MIDDAY ___ PM				
DEPTHS	SURFACE				BOTTOM		SURFACE				BOTTOM
TEMPERATURE											
pH											
SALINITY (CONDUCTANCE)											
DISSOLVED OXYGEN (mg/L)											

WEATHER CONDITIONS

PRECIPITATION *(amount)*	SKY *(percent cloud cover)*	AIR TEMPERATURE	WIND DIRECTION	VELOCITY

PRIOR WEATHER CONDITIONS *(3-4 days)*

SPECIAL CONDITIONS *(i.e. tide, drought, flood, hurricane, hot spell)*

OTHER COMMENTS

AEHA Form 29, 1 Apr 80 (HSE-EW)

FISH AND INVERTEBRATE OBSERVATIONS

		NO		YES
ANY VISIBLE SIGNS OF INJURY OR DISEASE? *(i.e. fungus, cyst)* *If yes, describe condition*		NO		YES
ARE ORGANISMS DYING AT PRESENT TIME?		NO		YES
ARE FISH SWIMMING WILDLY?		NO		YES
HAVE THE FISH LOST THEIR EQUILIBRIUM?		NO		YES
ARE THE FISH LETHARGIC?		NO		YES
ANY UNUSUAL BREATHING RATE? *(i.e. rapid, slow)* *If yes, describe*		NO		YES

CONDITION OF GILLS *(describe)*

INTERNAL ABNORMALITIES *(describe)*

KILL ESTIMATE

SPECIES	EST. NO. KILLED	SIZE RANGE	AVE. SIZE	AVE. WEIGHT	EST. TOTAL WEIGHT	COMMENTS

DURATION OF KILL: ☐ IN PROGRESS ☐ FINAL DETERMINATION (days, hours)

SUSPECTED CAUSE OF KILL:

☐ NATURAL ☐ MAN-CAUSED *(specify below)*

☐ AGRICULTURAL ☐ INDUSTRIAL ☐ MUNICIPAL ☐ OTHER

SPECIFIC AGENT OR CAUSE *(if known)*:

TYPE OF SAMPLES COLLECTED [*check appropriate box(es)*]

WATER SAMPLES		BIOLOGICAL SAMPLES
☐ BIOASSAY (2 GAL) ☐ CHEMISTRY ☐ PESTICIDES	☐ SEDIMENT SAMPLES	☐ FISH AND INVERTEBRATE CARCASSES ☐ PLANKTON

[redacted]	CERTIFIED BY *(signature and date)*

FISH AND INVERTEBRATE KILL EVALUATION FORM FOR FIELD INVESTIGATION *(USAEHA TECHNICAL GUIDE 116)*	INVESTIGATION DATE	INVESTIGATION TIME

INVESTIGATOR *(name)*	ORGANIZATION

AGENCIES NOTIFIED

FIELD REPRESENTATIVES PARTICIPATING FROM VARIOUS AGENCIES *(names)*

LOCATION

MAJOR WATER BODY AFFECTED	TRIBUTARIES INVOLVED	AREA AFFECTED	
		ACRES	RIVER MILES

GENERAL DESCRIPTION OF AREA

WATER CONDITIONS *(within KILL Zone)*

WATER CLARITY	UNUSUAL COLORATION	DEPTH	
VERY CLEAR ☐ CLEAR ☐ TURBID ☐ HIGHLY TURBID ☐	☐ NO ☐ YES *(state color)*	MEAN	MAXIMUM

UNUSUAL APPEARANCE *(i.e. algal blooms, oil, turbid)*

TIDAL DATA

PROFILES

	DAWN ___ AM						MIDDAY ___ PM				
DEPTHS	SURFACE				BOTTOM		SURFACE				BOTTOM
TEMPERATURE											
pH											
SALINITY (CONDUCTANCE)											
DISSOLVED OXYGEN (mg/L)											

WEATHER CONDITIONS

PRECIPITATION *(amount)*	SKY *(percent cloud cover)*	AIR TEMPERATURE	WIND DIRECTION	VELOCITY

PRIOR WEATHER CONDITIONS *(3-4 days)*

SPECIAL CONDITIONS *(i.e. tide, drought, flood, hurricane, hot spell)*

OTHER COMMENTS

AEHA Form 29, 1 Apr 80 (HSE-EW)

FISH AND INVERTEBRATE OBSERVATIONS

ANY VISIBLE SIGNS OF INJURY OR DISEASE? *(i.e. fungus, cyst)* *If yes, describe condition*		NO		YES
ARE ORGANISMS DYING AT PRESENT TIME?		NO		YES
ARE FISH SWIMMING WILDLY?		NO		YES
HAVE THE FISH LOST THEIR EQUILIBRIUM?		NO		YES
ARE THE FISH LETHARGIC?		NO		YES
ANY UNUSUAL BREATHING RATE? *(i.e. rapid, slow)* *If yes, describe*		NO		YES

CONDITION OF GILLS *(describe)*

INTERNAL ABNORMALITIES *(describe)*

KILL ESTIMATE

SPECIES	EST. NO. KILLED	SIZE RANGE	AVE. SIZE	AVE. WEIGHT	EST. TOTAL WEIGHT	COMMENTS

DURATION OF KILL: ☐ IN PROGRESS ☐ FINAL DETERMINATION (days, hours)

SUSPECTED CAUSE OF KILL:

☐ NATURAL ☐ MAN-CAUSED *(specify below)*

☐ AGRICULTURAL ☐ INDUSTRIAL ☐ MUNICIPAL ☐ OTHER

SPECIFIC AGENT OR CAUSE *(if known)*:

TYPE OF SAMPLES COLLECTED [*check appropriate box(es)*]

WATER SAMPLES

☐ BIOASSAY (2 GAL)
☐ CHEMISTRY
☐ PESTICIDES

☐ SEDIMENT SAMPLES

BIOLOGICAL SAMPLES

☐ FISH AND INVERTEBRATE CARCASSES
☐ PLANKTON

[redacted]

CERTIFIED BY *(signature and date)*

FISH AND INVERTEBRATE KILL EVALUATION FORM FOR FIELD INVESTIGATION *(USAEHA TECHNICAL GUIDE 116)*	INVESTIGATION DATE	INVESTIGATION TIME

INVESTIGATOR *(name)*	ORGANIZATION

AGENCIES NOTIFIED

FIELD REPRESENTATIVES PARTICIPATING FROM VARIOUS AGENCIES *(names)*

LOCATION

MAJOR WATER BODY AFFECTED	TRIBUTARIES INVOLVED	AREA AFFECTED	
		ACRES	RIVER MILES

GENERAL DESCRIPTION OF AREA

WATER CONDITIONS *(within KILL Zone)*

WATER CLARITY	UNUSUAL COLORATION	DEPTH	
VERY CLEAR ☐ CLEAR ☐ TURBID ☐ HIGHLY TURBID ☐	☐ NO ☐ YES *(state color)*	MEAN	MAXIMUM

UNUSUAL APPEARANCE *(i.e. algal blooms, oil, turbid)*

TIDAL DATA

PROFILES

	DAWN ___ AM						MIDDAY ___ PM				
DEPTHS	SURFACE				BOTTOM		SURFACE				BOTTOM
TEMPERATURE											
pH											
SALINITY (CONDUCTANCE)											
DISSOLVED OXYGEN (mg/L)											

WEATHER CONDITIONS

PRECIPITATION *(amount)*	SKY *(percent cloud cover)*	AIR TEMPERATURE	WIND DIRECTION	VELOCITY

PRIOR WEATHER CONDITIONS *(3-4 days)*

SPECIAL CONDITIONS *(i.e. tide, drought, flood, hurricane, hot spell)*

OTHER COMMENTS

AEHA Form 29, 1 Apr 80 (HSE-EW)

FISH AND INVERTEBRATE OBSERVATIONS

ANY VISIBLE SIGNS OF INJURY OR DISEASE? *(i.e. fungus, cyst)* *If yes, describe condition*		NO		YES
ARE ORGANISMS DYING AT PRESENT TIME?		NO		YES
ARE FISH SWIMMING WILDLY?		NO		YES
HAVE THE FISH LOST THEIR EQUILIBRIUM?		NO		YES
ARE THE FISH LETHARGIC?		NO		YES
ANY UNUSUAL BREATHING RATE? *(i.e. rapid, slow)* *If yes, describe*		NO		YES

CONDITION OF GILLS *(describe)*

INTERNAL ABNORMALITIES *(describe)*

KILL ESTIMATE

SPECIES	EST. NO. KILLED	SIZE RANGE	AVE. SIZE	AVE. WEIGHT	EST. TOTAL WEIGHT	COMMENTS

DURATION OF KILL: ☐ IN PROGRESS ☐ FINAL DETERMINATION (days, hours)

SUSPECTED CAUSE OF KILL:

☐ NATURAL ☐ MAN-CAUSED *(specify below)*

☐ AGRICULTURAL ☐ INDUSTRIAL ☐ MUNICIPAL ☐ OTHER

SPECIFIC AGENT OR CAUSE *(if known)*:

TYPE OF SAMPLES COLLECTED [*check appropriate box(es)*]

WATER SAMPLES	☐ SEDIMENT SAMPLES	BIOLOGICAL SAMPLES
☐ BIOASSAY (2 GAL) ☐ CHEMISTRY ☐ PESTICIDES		☐ FISH AND INVERTEBRATE CARCASSES ☐ PLANKTON

CERTIFIED BY *(signature and date)*

FISH AND INVERTEBRATE KILL EVALUATION FORM FOR FIELD INVESTIGATION *(USAEHA TECHNICAL GUIDE 116)*	INVESTIGATION DATE	INVESTIGATION TIME

INVESTIGATOR *(name)*	ORGANIZATION

AGENCIES NOTIFIED

FIELD REPRESENTATIVES PARTICIPATING FROM VARIOUS AGENCIES *(names)*

LOCATION

MAJOR WATER BODY AFFECTED	TRIBUTARIES INVOLVED	AREA AFFECTED	
		ACRES	RIVER MILES

GENERAL DESCRIPTION OF AREA

WATER CONDITIONS *(within KILL Zone)*

WATER CLARITY	UNUSUAL COLORATION	DEPTH	
VERY CLEAR ☐	☐ NO	MEAN	MAXIMUM
CLEAR ☐	☐ YES *(state color)*		
TURBID ☐			
HIGHLY TURBID ☐			

UNUSUAL APPEARANCE *(i.e. algal blooms, oil, turbid)*

TIDAL DATA

PROFILES

	DAWN AM						MIDDAY ___PM				
DEPTHS	SURFACE				BOTTOM		SURFACE				BOTTOM
TEMPERATURE											
pH											
SALINITY (CONDUCTANCE)											
DISSOLVED OXYGEN (mg/L)											

WEATHER CONDITIONS

PRECIPITATION *(amount)*	SKY *(percent cloud cover)*	AIR TEMPERATURE	WIND DIRECTION	VELOCITY

PRIOR WEATHER CONDITIONS *(3-4 days)*

SPECIAL CONDITIONS *(i.e. tide, drought, flood, hurricane, hot spell)*

OTHER COMMENTS

FISH AND INVERTEBRATE OBSERVATIONS

ANY VISIBLE SIGNS OF INJURY OR DISEASE? *(i.e. fungus, cyst)* *If yes, describe condition*		NO		YES
ARE ORGANISMS DYING AT PRESENT TIME?		NO		YES
ARE FISH SWIMMING WILDLY?		NO		YES
HAVE THE FISH LOST THEIR EQUILIBRIUM?		NO		YES
ARE THE FISH LETHARGIC?		NO		YES
ANY UNUSUAL BREATHING RATE? *(i.e. rapid, slow)* *If yes, describe*		NO		YES

CONDITION OF GILLS *(describe)*

INTERNAL ABNORMALITIES *(describe)*

KILL ESTIMATE

SPECIES	EST. NO. KILLED	SIZE RANGE	AVE. SIZE	AVE. WEIGHT	EST. TOTAL WEIGHT	COMMENTS

DURATION OF KILL: ☐ IN PROGRESS ☐ FINAL DETERMINATION (days, hours)

SUSPECTED CAUSE OF KILL:

☐ NATURAL ☐ MAN-CAUSED *(specify below)*

☐ AGRICULTURAL ☐ INDUSTRIAL ☐ MUNICIPAL ☐ OTHER

SPECIFIC AGENT OR CAUSE *(if known)*:

TYPE OF SAMPLES COLLECTED [*check appropriate box(es)*]

WATER SAMPLES	☐ SEDIMENT SAMPLES	BIOLOGICAL SAMPLES
☐ BIOASSAY (2 GAL) ☐ CHEMISTRY ☐ PESTICIDES		☐ FISH AND INVERTEBRATE CARCASSES ☐ PLANKTON

CERTIFIED BY *(signature and date)*

APPENDIX C

SYMPTOMS EXHIBITED BY FISH EITHER PARASITIZED OR DISEASED

Symptoms Exhibited by Fish Either Parasitized or Diseased

Any one or combination of symptoms from the following three groups may indicate the presence of a disease or a parasite infestation.

I. Behavioral Characteristics

A. Nervous twitching of fins.
B. Flashing or darting.
C. Drooping fins.
D. Failure to feed.
E. Weakness - lethargy
F. Gather in vegetation.
G. Gather in shallow water.
H. Gather at incoming water.
I. Convulsions.
J. Unusual fin postures.
K. Gasping at surface.
L. Operculum (gill covering) with rapid movement.
M. Abnormal position in water.
N. Abnormal swimming movement - DESCRIBE

II. External Surface of Fish

A. Gills

1. Any color other than the normal red.
2. Parasites attached.
3. Hemorrhage present.
4. Abnormal morphology.
5. Excess mucus.

B. Eyes

1. Containing worms.
2. Cloudy.
3. Hemorrhage present.
4. Exophthalmos- "POP EYE".
5. Cotton like covering.

C. Fins

1. Hemorrhage or lesions present.
2. Cotton like covering.
3. Frayed or missing.
4. Parasites attached.

D. Body

1. Excessive mucus production.
2. Cutaneous lesions and hemorrhage present.
3. Color changes.
4. Emaciation.
5. Deformed - bent, twisted, rigid.

6. Diarrhea.
7. Swollen bellies.
8. Pustules or blisters.
9. Cotton like patches.

E. Scales

1. Loose patches.
2. Missing patches.

F. Mouth

1. Eroded or ulcerated.
2. Hemorrhage present.
3. Hyper-extended in death.
4. Cotton like patches.

III. <u>Internal Parts of Fish</u>

A. Muscle tissue

1. Hemorrhage or lesions present.
2. Other discoloration.
3. Grubs or worms present.

B. Body oavity

1. Body fluid any color other than clear.
2. Hemorrhage or lesions.
3. Air bladder hard, soft, partially filled.
4. Parasites present.

   a. In body cavity.
   b. In organs (i.e. liver, G.I. tract, etc.).

5. Gastro-intestinal tract

   a. Empty or full.
   b. Contents - what?
   c. Parasites present.

6. Liver

   a. Lesions present.
   b. Color - should be light brown.

7. Kidney

   a. Should not be spotted.
   b. Should be dark red to purple.

VISUAL SIGN	CAUSE/RECOMMENDATION

Found Externally

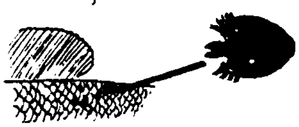
1. Fish popeyed; scales puffed with fluid (dropsy). Bloody wounds; blood under scales.

Various Bacteria such as Aeromonas sp.* Commonly found in water. Aeromonas normally does not infect fish unless they have undergone some stress. Fish with severe popeye or dropsy probably will not bite but can be seen dead or in distress along the shore. In some cases open bloody wounds can result from the bacterial infection.

2. Red pustule on or near base of fins; thread-like body may protrude from the wound.

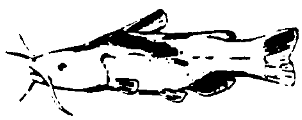
Anchor Worm (*Lernaea* sp.). This copepod buries only its anchor-shaped head into a fish's flesh. The remaining portion will hang free from the wound, where a red inflamed pustule may form. This parasite may drop off, leaving only the inflamed area.

3. Bloody area on body under the scales.



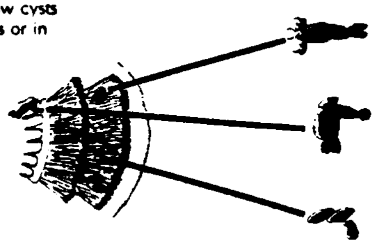
Fish Louse (*Argulus* sp.). This rarely seen copepod leaves a fish soon after it's removed from the water. It feeds on the blood by piercing the skin, destroying the protective mucous coat in the process. Thus, secondary infection from bacteria or fungus can result.

4. Tiny mobile white spots on the skin.



Ich (*Ichthyophthirius* sp.). The most common protozoan encountered by fishermen. Ich appears as mobile white spots or clusters on the skin or gills. It burrows under the skin and may cause surface lesions. Individuals can be seen with a magnifying glass.

5. White or yellow cysts or sacs on gills or in mouth.



A. (*Ergasilus* sp.). When numerous, these copepods can kill young fish. Their presence is indicated by V-shaped white egg sacs on the inner edges of the gills.

B. (*Achtheres* sp.) Larger than *Ergasilus*, this copepod attaches itself in the mouth or to the inner surface of the gills. *Achtheres* has a short plump body with armlike appendages that cling to the fish.

C. **Yellow Grub** (*Clinostomum* sp.). This larval fluke forms cream-colored cysts on the gills and under the skin in the mouth. It can easily be seen with a magnifying glass if cyst is broken.

6. White pustules under skin or scales.

(Myxosporidia). The white cysts created by Myxospondia hold thousands of the microscopic protozoans. While certain species cause some important diseases in fish, none have been found in Nebraska.

7. Patches of fuzzy grey-white mat on body and gills.

Water Fungus (*Saprolegnia* sp.). Usually found on fish injured by improper handling or other cause. When established, Water Fungus can kill a fish by completely covering it.

8. Grey-white slime on the skin.

Columnaris Disease (*Condrococcus columnaris*). This bacterial infection may be found on catfish, trout, and possibly other species. Frayed fins and bloody wounds are other indicators.

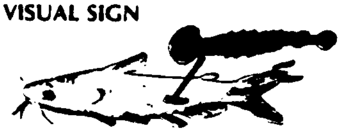
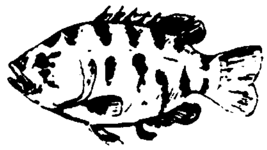
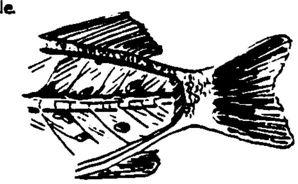
9. Black spots under the skin or in the flesh.

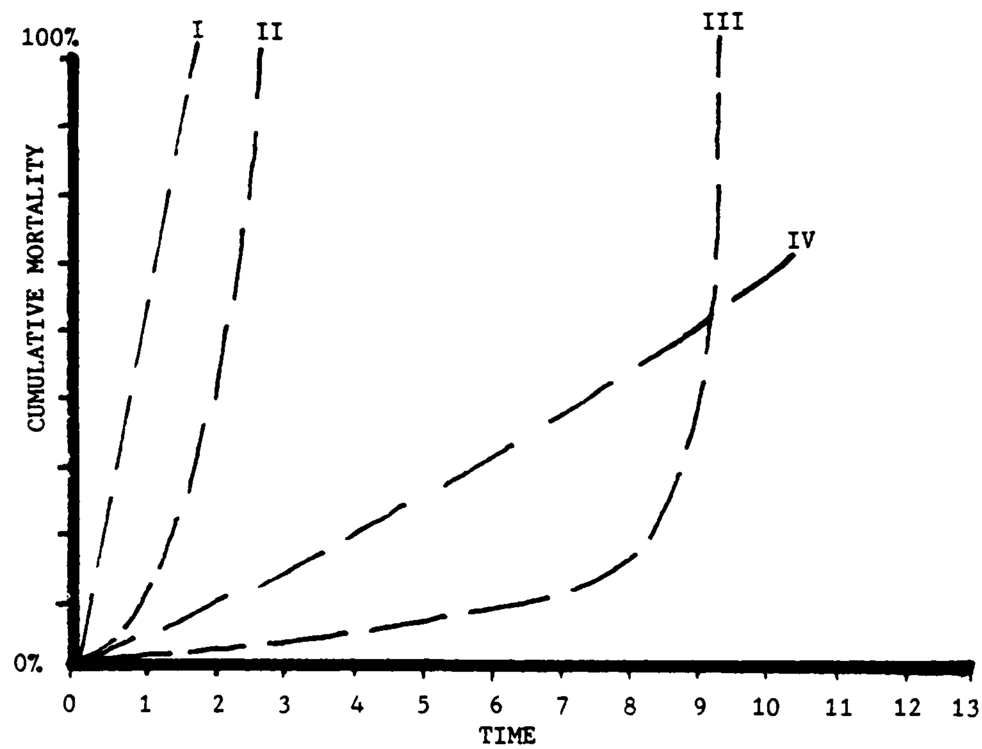
Black Spot (*Neascus* sp.). The easiest disease to recognize. Black Spot is caused by larval flukes burrowing under the skin. Appearing as small round black spots, the cysts may also be found in the flesh.

10. Eye deformed; fish apparently blind.

Eye Fluke (*Diplostomulum* sp.). These tiny larval flukes will not be seen. They live in the fluid of the eye and eventually cause blindness. Eye may be opaque or shrunken.

* sp = species

VISUAL SIGN		CAUSE/RECOMMENDATION
11. Undulating worms attached to body, fins, gills, and mouth.		**Leeches.** Conspicuous, blood-feeding, external parasites, leeches produce a small circular wound that remains even though the leech moves or drops off.
12. Red, thread-like worms extending from the anus.		**Round Worms** (*Camallanus* sp.). Various roundworms are found throughout the intestine. The species that lives in the lower large intestine will occasionally extend from the anus. [redacted]
13. White to pink thread-like swelling on head or fins.		**Round Worms** (*Philometra* sp.). Normally found on carp, buffalo, and suckers, this adult roundworm lives just under the skin.
Found in the Flesh		
14. White or yellow cysts imbedded in the muscle.		**Yellow Grub** (*Clinostomum* sp.). Cream-colored cysts found in many parts of the body contain larval flukes that become adults in birds. Numerous at times, the Yellow Grub will emerge if cyst is broken in water.
		White Grub (*Hysteromorpha* sp.). Smaller and lighter colored than the Yellow Grub. These larval flukes are most often found in catfish.
15. Sandy flesh in walleye.		**Unknown.** An unusual problem apparently found only in walleye. Fish show no external symptoms or abnormal behavior. The rough, sandy flesh is found in varying intensity when fish is filleted but the flesh is always somewhat discolored.
Found Internally		
16. Large white flat worm in the body cavity.		**Tapeworm** (*Ligula* sp.). This larval tapeworm is found free in the body cavity of minnows, carp, suckers, and some other fish. It is uncommonly large and may create an abdominal bulge.
17. Coiled (like a watch spring) worm encysted on the internal organs.		(*Contracaecum* sp.). Found on the internal organs or the wall of the body cavity, these larval roundworms are immobile. They become adult in fish-eating birds. [redacted]
18. Round transparent cysts on the internal organs.	 Heart	**White Grub** (*Neascus* sp.). These larval flukes occasionally occur in quite large numbers.
19. Irregular white cysts in or on the internal organs.		**Larval Spiny-Headed Worm or Larval Tapeworm.** These cysts are larger, whiter, and not as round as those described in No. 18.
20. White, thread-like worms lying on or moving through the internal organs.		**Larval Tapeworm.** Some tapeworms are not found in cysts. Numerous worms may infect the ovaries of bass.
21. Tiny gold-brown cysts on the internal organs.	 Liver	**Larval Roundworm.** Often found in great numbers, these cysts will give a sandy appearance to a fish's innards.
22. White or orange worm in body cavity, attached to the intestine.		**Spiny-Headed Worm** (*Pomphorhynchus* sp.). Since most adult acanthocephalans live inside the intestine they are not seen by fishermen. However, this species can be found lying in the body cavity with its head buried in the intestine.
23. White, undulating worms emerging from ruptured intestine.		**Intestinal Worms (Adult Helminths).** Adult flukes, tapeworms, roundworms, and spiny-headed worms will not normally be seen by fishermen unless the intestine is accidentally cut by cleaning.



I. Very sudden die-off (Causative agent usually environmental i.e. pH, DO, etc; pesticides or other chemical agents)

II. Slow starting followed by rapid die-off (Causative agent usually viral or very virulent bacteria or other pathogen - usually no lesions present on fish)

III. Slow die-off for several days or weeks followed by a rapid die-off (Causative agent usually a synergistic action of combinations of numbers I, II, and/or IV)

IV. Slow gradual die-off. Only a few deaths daily (Causative agent usually low virulence bacteria, external parasites, or marginal environmental conditions -- lesions usually present on fish)

APPENDIX D

RECOMMENDATIONS FOR SAMPLING AND PRESERVATION OF SAMPLES

TABLE D-1. PRESERVATION GROUPS FOR WATER ANALYSES

Listed below are typical water analyses USAEHA could conduct in the event of a fish kill. They are grouped according to maximum holding time, preservation requirements, container type, and sample volume. These requirements are presented in Table D-2.

PRESERVATION GROUP A

Color
Nitrate-Nitrogen
Nitrite-Nitrogen
Sulfite
Surfactants
Turbidity
Phosphorous-Ortho (filtered and keep in separate 250 mL bottle)

PRESERVATION GROUP B

Acidity
Alkalinity
Chloride
Residue, Total
Specific Conductance
Sulfate

PRESERVATION GROUP C

Ammonia
Kjehldahl and Organic Nitrogen
Nitrate and Nitrite-Nitrogen
Organic Cabron
Phenols
Phosphorous, Total

PRESERVATION GROUP D

Oil and Grease

PRESERVATION GROUP E

Cyanide, Total

PRESERVATION GROUP F

Aluminum
Cadmium
Chromium
Hardness
Copper
Iron
Lead
Nickel
Silver
Zinc and Others

PRESERVATION GROUP G

Mercury

PRESERVATION GROUP H

Pesticides/Herbicides/PCB's/Organics

TABLE D-2. SAMPLE VOLUME, HOLDING TIME, AND PRESERVATION REQUIREMENTS FOR THE PRESERVATION GROUPS GIVEN IN TABLE D-1

Group	Minimum Volume(1)	Container Type(2)	Preservation(3)	Holding Time
A	1,000 mL	P,G	Cool, 4°C	48 hours
B	1,000 mL	P	Cool, 4°C	7 days
C	250 mL	P	Cool, 4°C, H_2SO_4 to pH>2 or add 0.5 mL 1:1 sulfuric acid(4) to 250 mL sample	28 days
D	1,000 mL	G	Cool, 4°C, H_2SO_4 to pH>2 or add 2 mL 1:1 sulfuric acid to 1,000 mL sample	28 days
E	250 mL	P,G	Cool 4°C, NaOH to pH <12 (Add 40% NaOH solution dropwise to pH 12)	14 days
F	250 mL	P,G	HNO_3 to pH>2 or add 1 mL 1:1 nitric acid(4) to 250 mL sample	6 months
G	125 mL	P,G	HNO_3 to pH>2, 0.05% $K_2Cr_7O_5$ or add 0.2 mL of 0.1% $K_2Cr_7O_5$ in 0.5% HNO_3 solution	28 days
H	1,000 mL	G(5)	Sediments, cool 4°C	7 days
	2,000 mL	G(5)	Pesticides, cool, 4°C	7 days
	2,000 mL	G(5)	Organics, cool, 4°C	7 days

(1) If samples are to be analyzed by a laboratory other than USAEHA, 1000 mL (1 qt) should be collected wherever 250 mL (8 oz) is listed.
(2) New polyethylene (P) or clean glass (G)
(3) Do not add preservative if it will cause an adverse or unsafe reaction with the sample, especially with industrial process samples; for example, in cyanide plating solution DO NOT add sulfuric acid. Contact USAEHA for guidance in these situations.
(4) 1:1 acid solution. Mix equal volumes of concentrated acid (sulfuric or nitric) and distilled water (add acid to water).
(5) The bottles should be rinsed with pH-2 sulfuric acid water, distilled water-rinsed, acetone-rinsed, air-dried, and capped using a Teflon or aluminum foil liner (dull side toward sample).

APPENDIX E

BASIC COUNTING PROCEDURES FOR INVESTIGATING FISH KILLS

PART I

BASIC COUNTING PROCEDURES FOR INVESTIGATING FISH KILLS IN STREAMS

A 100-yard count is made every one-half mile beginning with a randomly-chosen site within the first half-mile section of kill area. The first count is randomly chosen by a series of two coin tosses. The first toss determines whether the count will be made within the first or last quarter-mile of the first half-mile of kill area. The second toss determines whether the count will be within the first or last 100-yards of the previously determined quarter-mile section.

The additional counts should then be made at each half-mile interval throughout the region of the kill. If access limitations make exact, half-mile intervals difficult, approximate intervals can be determined taking advantage of access points. However, be sure that the intervals are evenly spaced including at least one counting section within each successive half-mile kill area. If access points rather than measured half-mile intervals are used, randomness of selection of counting section must be insured. Therefore, it is arbitrarily suggested that, in such cases, the investigator begin the 100 yard count 40 yards above the access point and proceed upstream.

The count will consist of the following steps:

1. Identify, count, and determine inch groups of all fish in each 100-yard segment.
2. By using a map and map measurer, determine the exact length of the kill area if not done during the field investigation. Divide the total number of yards counted (add all 100-yard segments counted) into the total length of the kill for an exact ratio of fish counted to total fish killed. This is the expansion factor.
3. Multiply the total number of each size group of each species by the expansion factor arrived at in Item 2. These figures represent the total estimated numbers killed.

In order to facilitate use of this method an example is included below:

	Species	Number	Inch Group
1st 100 yards	Bluegill	140	1
		120	2
		60	3
		30	4
		25	5
		30	6
		10	7
		5	8
		420	
2nd 100 yards	Bluegill	100	1
		80	2
		40	3

	Species	Number	Inch Group
2nd 100 yards	Bluegill	20	4
		15	5
		15	6
		5	7
		5	8
		280	
3rd 100 yards	Bluegill	40	1
		30	2
		20	3
		15	4
		25	5
		20	6
		10	7
		5	8
		165	
4th 100 yards	Bluegill	0	1
		10	2
		5	3
		10	4
		15	5
		5	6
		0	7
		0	8
		45	
Total Bluegills counted		910	

Calculated total length of kill - 2 miles (3,520 yards)

Expansion factor $\frac{\text{3,520 yards (total length of kill)}}{\text{400 yards (total number of yards counted)}} = 8.8$

Total Bluegills killed 8.8 x 910 =8,008

Counts such as those illustrated should be made for each species and the total number of fish killed calculated. Since the kill will be assessed by use of the monetary values, it is necessary to have a breakdown to the various inch group categories. In the event of excessive large kills over many stream miles the investigator may deem it necessary to make counts at one mile intervals.

PART II

BASIC COUNTING PROCEDURES FOR INVESTIGATING FISH KILLS IN LAKES

First, the overall limits of the kill in the lake should be determined by cursory inspection. For purposes of counting, the kill should be divided into two subsamples: (1) those windrowed near shore, or otherwise accumulated along the shoreline; and (2) those found in open water. Figures obtained from each of these subsamples will be expanded independently and added for a total sum of fish killed. Fish should be identified and sized in the same manner described in the stream counting procedure. This method is applicable to wide, navigable streams as well as lakes.

1. Shoreline Count: The bulk of the dead fish (usually over 75%) will be found along the shoreline. The principle of the counting method here is similar to that for stream kills. Count a 300-foot length of shoreline per 1/2 mile of shoreline included in the kill. A minimum of three shoreline counts should be made. If the body of water is linear in such a way that the kill area is or resembles a wide stream, the first count should begin where the first dead fish occurs. In this situation, counts should be made on both sides of the "stream". If an irregularly shaped body of water is involved, the first count should be determined arbitrarily. The width of the 300-foot counting strips should be consistent but may be the choice of the investigator. Expand the sample figures obtained in the same manner as described in the stream counting procedure.

2. Open Water Count (see figures 1 and 2): The principle to be used will involve transect counts of a given width, each transect count being taken a given constant distance from and parallel to the next. Make transect samples at approximately 300-foot intervals throughout the length of the kill (T). This interval (w + y) may have to be lengthened for large kills. The width (w) of each sample should be constant but may be the choice of the investigator. A total of 20 feet is practical (10 feet either side of center of boat) for the width (w). Following is a table showing % area counted using various intervals and a 20-foot transect width (w).

	w(ft.)	interval(ft.) (w + y)	% fish counted
	20	200	10.0
Recommended interval	20	300	6.7
	20	600	3.3
	20	900	2.2
	20	1200	1.7
	20	2000	1.0

A bar extending to both sides of the boat and spanning the sample width (w) would be helpful in delineating the sample area as the investigator crosses the lake. The length (1) of the transects is the distance from shore to shore minus the width of shoreline count strips at each shore. You need only a total tally of fish counted. There is no need of keeping individual transect counts separate. The first count is made at the point along distance (T) where the first fish is found or otherwise chosen arbitrarily. Depending on the shape of the lake, two approaches may be applied in completing the open water count:

A. The first approach applies best to wide streams, main stream reservoirs, and lakes of relatively constant breadth (see Figure 1). Under these conditions, the transect length (l) need not be determined. The total length of the kill (T) need not be determined until after the counting procedure. Simply count transects at estimated constant intervals (e.g. w + y = 300 ft.), keeping track of the number of transects you counted. The number of transects made should afterwards be divided into total kill length (T/No. of transects made) for a check of your actual, average interval (w + y). Then, proceed to calculate total estimated fish in open water.

$$\frac{w + y \text{ (actual)}}{w} \times \begin{array}{c}\text{Total fish}\\ \text{counted}\end{array} = \text{Total fish in open water}$$

EXAMPLE

Width of transect (w) = 20 feet
Estimated sampling interval (w + y) = 300 feet

Estimating 300-foot intervals, you count a total of 540 fish in 19 transects.
Checking later, you find from notes and a map that the kill area is one mile long. A 300-foot interval would only call for 17.6 transects in a mile, so you must determine your actual interval for purposes of making calculations:

$$\text{Actual (adjusted) interval } (w + y) = \frac{5{,}280}{19} = 278 \text{ feet}$$

$$\text{Total fish in open water} = \frac{278}{20} \times 540 = 7{,}506$$

B. Odd-shaped lakes and storage reservoirs do not lend themselves to the above method because of extreme variances in the length of transects (l). In these lakes (see Figure 2) where transects would not average a fairly constant length (l), area should be the basis of computation. With this approach, the length (l) of the transects needs to be determined from a map as well as the acreage of the kill area. The following computations will yield the open water estimate on a total, and per acre basis:

1. $$\text{Acreage sampled} = \frac{w \times (l_1 + l_2 + l_3 \cdots\cdots + l_{(\text{no. of transects})})}{43{,}560}$$

2. $\text{Acreage considered in shoreline sampling} = \dfrac{\text{Feet of shoreline X width of shoreline sample strip}}{43{,}560}$

3. Open water acreage = Total acreage - acreage considered in shoreline sampling (2)

4. $\text{Fish dead/acre open water} = \dfrac{\text{Total fish counted}}{\text{acreage sampled (1)}}$

5. $\text{Total fish dead in open water} = \text{Fish dead/acre open water (4)} \times \text{Open water acreage (3)}$

EXAMPLE (see Figure 2):

Fish were counted in transects at about 300-foot intervals in a 13.1 acre lake. 300 fish were counted in total.

w = 20 feet
Number of transects = 6
1_1 400 ft.
1_2 800 ft.
1_3 1000 ft.
1_4 900 ft.
1_5 850 ft.
1_6 600 ft.

1. $\text{Acreage sampled} = \dfrac{20\ (400+800+1000+900+850+600)}{43{,}560} = 2.089 \text{ acres}$

2. $\text{Acreage considered in shoreline} = \dfrac{7000 \text{ ft.} \times 10 \text{ ft.}}{43{,}560} = 1.6$

3. Open water acreage = 13.1 - 1.6 = 11.5 acres

4. $\text{Fish dead/acre open water} = \dfrac{300}{2.089} = 144 \text{ fish}$

5. Total fish dead in open water = 144 x 11.5 = 1,656 fish

Figures obtained from open water estimate are added to those from the shoreline estimate for a total number of fish killed in the lake.

FIGURE 1

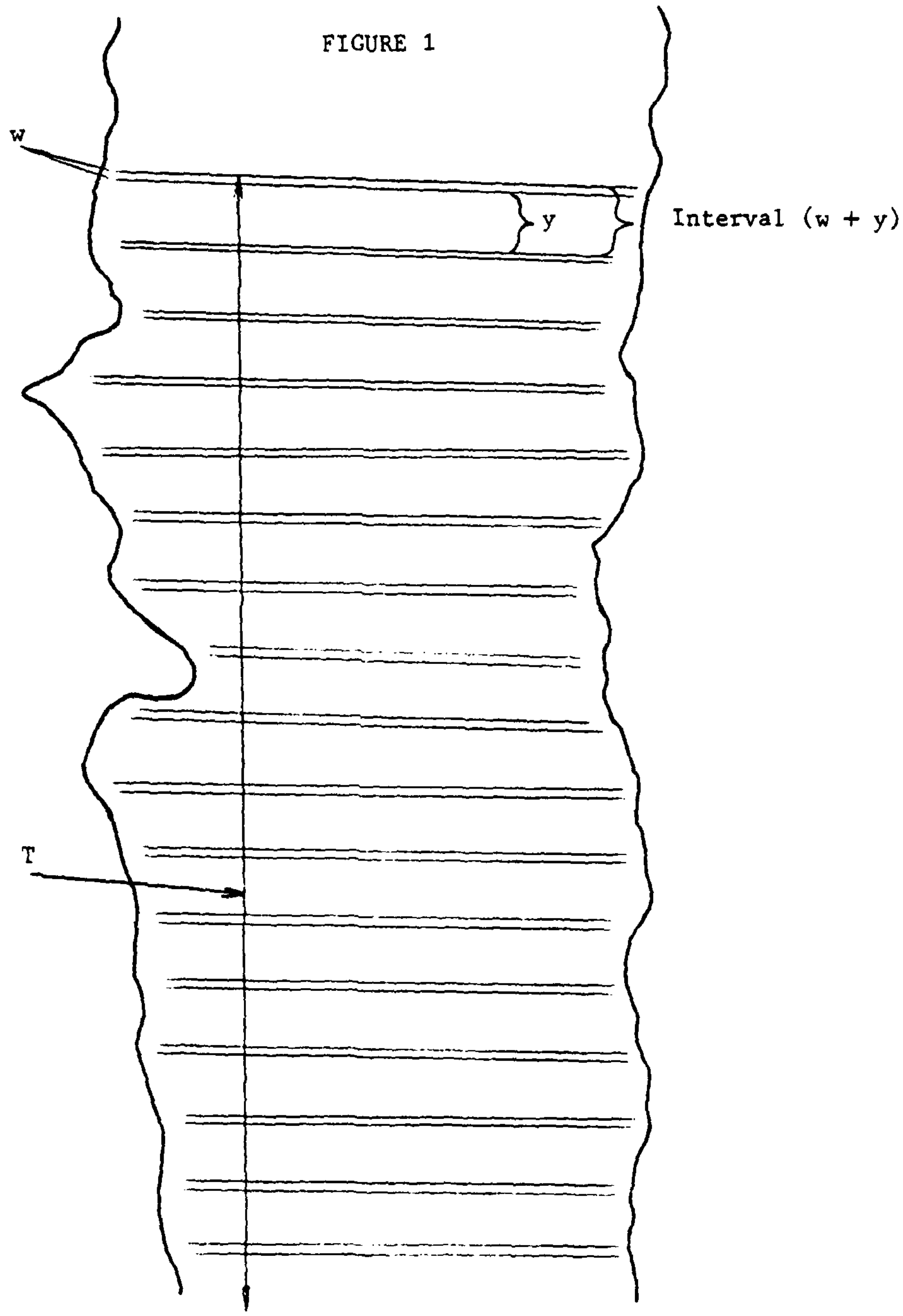
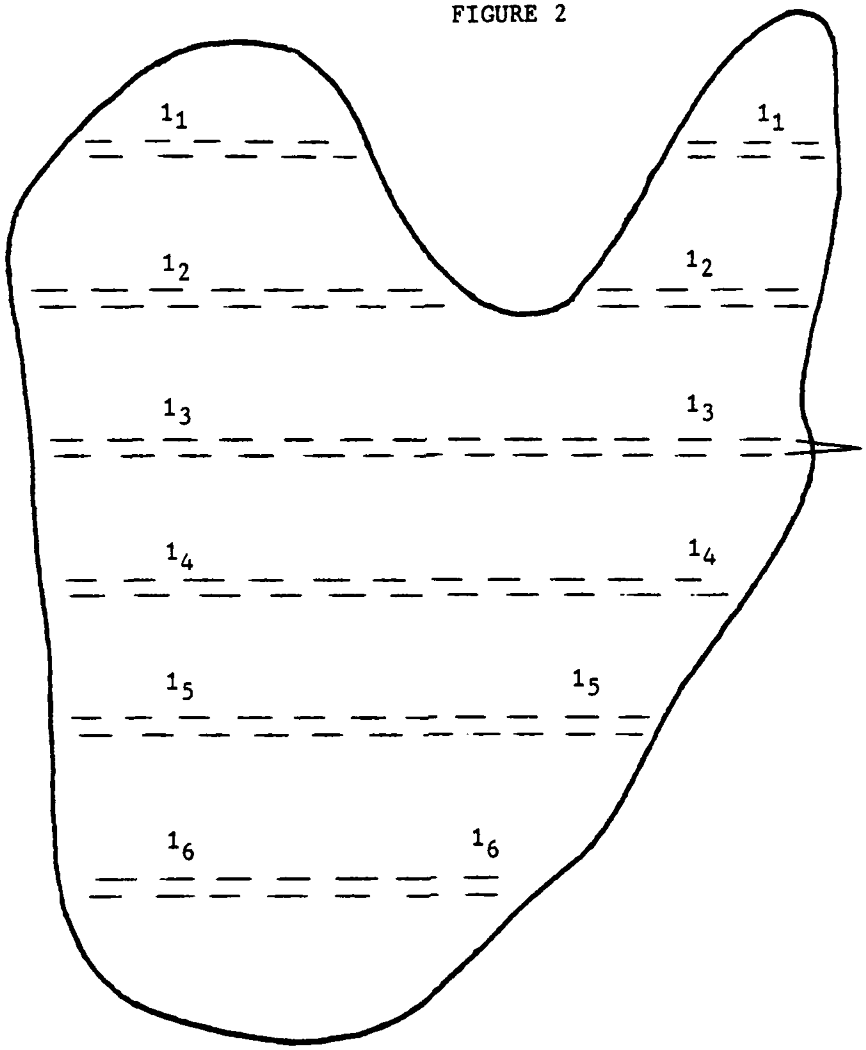


FIGURE 2



APPENDIX F

GLOSSARY

BOD	Biochemical Oxygen Demand - the amount of oxygen required as a result of microbial decomposition usually for 5 days at 20°C in water
COD	Chemical Oxygen Demand - the amount of oxygen required to oxidize the chemical constituents in water
DO	Dissolved Oxygen - the amount of oxygen dissolved in water
EPA	US Environmental Protection Agency
Hypolimnion	the cold, dense water below the thermocline in a water body
Necropsy	the examination of a dead body, including dissection; a post mortem examination; an autopsy
Parasite	an organism that lives on or in another organism (the host) and receives benefit (such as food) while causing harm to the host
PCB	Polychlorinated Biphenyl - a highly toxic and accumulative organic compound
Seiche	a wave that oscillates in lakes, bays or gulfs as a result of seismic or wind disturbance
Thermocline	the layer of water in which temperature change is rapid, causing a density barrier between warm surface water and the cold hypolimnion
Ubiquitous	being everywhere
USAEHA	US Army Environmental Hygiene Agency

APPENDIX G

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